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STUDIES ON INSECT SPERMATOGENESIS. III. ON THE STRUCTURE OF THE NEBENKERN IN THE INSECT SPERMATID AND THE ORIGIN OF NEBENKERN PATTERNS.

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INTRODUCTION.

In the preceding "Study" of this series I have given a rather full account of the formation of the hemipteran spermatid together with a history of its various components in the subsequent transformation into the mature sperm. An outline of the behavior of the mitochondria (nebenkern) was included in that account, but it was thought best to postpone any extended consideration of details to a later paper. It is the purpose of this paper to complete the description of the nebenkern especially with respect to the complicated and fantastic "patterns" which are its chief characteristic in the insect spermatid.

By the term "pattern," I refer to those curious appearances, which have been likened to a blackberry, an onion, a ball of twine, a skein of yarn, a spireme, and other similar objects, which develop in the nebenkern of the early spermatids and eventually disappear again. It is rather disconcerting to find that after thirty-five years of observation these appearances are not yet understood—have never, indeed, been carefully examined as to origin and significance except by Gatenby, whose recent work on the Lepidoptera has served me as a point of departure for this paper. I have not been able to find with certainty who first noted these patterns—the early workers seem to have taken them as a matter of course and to have given them no particular emphasis.

The earliest clear account which I have discovered is that of von la Valette St. George ('86*a*), who clearly figured the "onion" stage in *Blatta* (see his Fig. 74), and likened it to a ball of thread. Later, he described much the same appearance in *Phratora* ('86*b*) and (doubtfully) in *Forficula* ('87). These accounts were the forerunner of a long series of similar observations in which the distinctive feature of nebenkern structure is a more or less thread-like appearance, or more correctly, a division of its substance into layers which are often likened to those of a hemi-sected *onion*. Another characteristic appearance, occurring often times in conjunction with the preceding, was first described by Platner ('89), who noted the differentiation of the nebenkern in Lepidoptera into two substances concentrically arranged—an inner, darkly stained core enclosed in an outer clear, non-staining material. Subsequent observers have found in many forms a similar peripheral zone, often broken up into vacuoles by thin partitions traversing the clear substance. Still a third type of pattern has been described particularly by Holmgren ('02) in *Silpha,* and by Vejdovský ('12) in *Diestrammena,* consisting of a series of smooth or beaded cords running lengthwise through the nebenkern as it begins its elongation. This appearance seems to have been seen by comparatively few observers, and to have been misinterpreted by all. The problem is, therefore, a twofold one: (1) what is the structural basis of these patterns? and (2) what is the relation of these various appearances to each other and to the course of sperm formation as a whole? In the account which follows, I have tried to give at least a descriptive answer to these questions.

The material for this "Study," as in the preceding ones, has been drawn from Hemiptera belonging to the Family *Pentatomidæ*. As I have already pointed out (Bowen, '20), this particular family is characterized by the occurrence of numerous species in which certain of the spermatocytes and spermatids are unusually large as compared with the remaining ones, which are strikingly smaller ("normal") in size. The occurrence of these *large* spermatids, with *correspondingly large nebenkerns,* has made possible a structural analysis of the nebenkern which

would have been exceedingly difficult, if not entirely impossible, in the spermatids of "normal" size. This paper, therefore, deals exclusively with the large-cell generations, but the results have been checked in the small-cell generations and it is clear that the phenomena are in both cases essentially the same, the factor of size being the sole difference. I have used for this study three forms—*Euschistus euschistoides* Voll., *Brochymena quadripustulata* Fab., and *Murgantia histrionica* Hahn, each of which possesses two testicular lobes of large-cell generations.

I have made use particularly of three technical methods—Benda's alizarin-crystal violet stain and the Golgi methods of Kopsch and Cajal, the reasons for employing these particular methods being made evident by the descriptive section which follows. The first named was used exactly as described in the second of these "Studies." The Kopsch method does not usually impregnate the mitochondria, but occasionally the nebenkern and its derivatives are blackened—in the present case after an immersion in 2 per cent. osmic acid of about ten days. The Cajal formol-uranium nitrate method was used as described by Cajal,[1] with a primary fixation of 8 to 10 hours, followed by silver nitrate (1.5 per cent.) for 36 or 47 hours, and reduction with hydroquinone for periods up to 24 hours. At best this method is a rather capricious one.

I have here to acknowledge the many helpful suggestions and criticisms which have been contributed from time to time by Professor E. B. Wilson. I am also indebted to Dr. Franz Schrader for assistance in the collection of material, and particularly to Mr. H. G. Barber through whose unusual kindness I was able to get the specimens of *Brochymena* which I have used in this "Study." Mr. Barber has also identified all of my material.

Observations and Discussion.

In an earlier work (Bowen, '22), I have given a detailed account of the manner in which the spermatid nebenkern is condensed from the originally thread-like (with some inter-mixed

[1] See Cajal, Algunas variaciones fisiológicas y patológicas del aparato reticular de Golgi., Trab. Lab. Biol. Univ. Madrid, 1914, Vol. 12.

granules) mitochondria into a compact, spherical chondriosome body. In the large spermatids this body usually appears, immediately after formation, of rather homogeneous texture, staining faintly or not at all with crystal violet. In the small spermatids the nebenkern typically stains an intense violet (after Benda). Thus, if there is any structural differentiation of the nebenkern in this initial stage it is lost in one case by failure to stain and in the other by over-staining. Subsequent phenomena indicate, however, that there must be at this time some differentiation of the mitochondrial substance, and it will first be necessary to examine carefully the evidence bearing on this point.

As other observers have remarked, the mitochondria in the spermatocytes at the time of the maturation divisions appear characteristically in the form either of granules or of rods and threads of various dimensions. As Meves ('00) was the first to show (in *Pygæra*), the granular mitochondria are not homogeneous spheres, but are actually vesicles the substance of which is differentiated into two distinct parts—an intensely staining envelope or peripheral layer and a clear, non-staining interior. This structure is very clearly demonstrated in many other Lepidoptera, as recently shown by Gatenby ('17), who has applied the convenient descriptive adjective *chromophilic* and *chromophobe* (or better, *chromophobic*), to the staining and non-staining portions respectively of the chondriosome vesicles. The same vesicular structure has also been shown to occur in some forms other than insects (*e.g.*, Mollusca), so that it would appear reasonable to expect a similar duplex structure in mitochondria of the rod type. The evidence here is not entirely convincing, due largely to the difficulties of technique. Meves ('07) has, however, noted that in the thread-like mitochondria of the honey-bee, there is a differentiation into an outer, chromophilic envelope with a core of non-staining material. Gatenby ('18) has recently stated that in the beetle (*Tenebrio*) the mitochondria occur either as "spheres" or as "elongated tubes, containing internally a chromophobic substance." In the Hemiptera, I have never been able to satisfy myself of this duplex structure. The mitochondria are in the form of long, delicate threads (Bowen, '20) which are so fine that

it has not yet been possible to distinguish any internal structural features. However, in one case, *Murgantia histrionica,* I have found that the mitochondria at an early spermatocyte period pass through a granular stage in which it is possible clearly to make out the same differentiation into two substances which others have noted in the like granular mitochondria of Lepidoptera. Subsequently the granules produce the long, thread-like mitochondria typical of *Euschistus* and other pentatomids. It is, I think, reasonable to presume that the structure of the granules is carried over into the threads, which would thus correspond in structure with those of *Tenebrio.* Without going further into these purely structural aspects, it seems to me fair to conclude that in the male germ cells at least the mitochondria are generally composed of two distinct substances, related to each other as I have described above. Whether there is any relation between this morphological duplicity and the presumed chemical composition (lipoid plus albumenoid) of the mitochondria is entirely problematical. Indeed, it is not unlikely that our ideas of the chemical nature of these bodies will have to be revised in the light of recent cytological investigations.

Assuming, then, a duplex morphological structure for the mitochondria, we are now in position to take up the structure of the nebenkern at the time when it has just rounded out into its typical shape. Here again, our problem is simplified by a preliminary study of the formation of a nebenkern from the granular type of mitochondria. This was first observed in detail by Meves ('00) in *Pygæra,* and more recently the same ground has been worked over by Gatenby ('17) in a most illuminating manner. Meves showed that at the close of the second maturation division, the vesicular mitochondria begin to fuse together into an irregular, darkly staining mass containing many large, non-staining vacuoles. Subsequently, the stained substance is aggregated into a roughly spherical mass enclosed in a non-staining envelope which is very sharply delimited externally and is traversed by numerous strands passing out from the central, chromophilic core. These latter disappear eventually, and the nebenkern is smoothed out into the two zones observed many years before by

Platner ('89). Meves concluded that in the process of condensation, the constituent materials of the original mitochondrial vesicles had simply exchanged places, the chromophilic substance having run together to form a homogeneous, central core, for which the formerly internal, chromophobic substance now acts as an envelope.

Gatenby's ('17) account[1] is much more complete and proves conclusively that the details as given by Meves are badly distorted through the use of acetic acid in the fixative. Gatenby shows that the running together of the mitochondrial vesicles occurs in a rather orderly way, the chromophobic material gradually fusing to form the main body of the nebenkern within which the chromophilic substance is molded into a long, but perfect spireme wound up, as it were, within the nebenkern proper. Omitting, for the present, any discussion of details, it is clear that the nebenkern thus formed is not homogeneous, but quite the contrary, the exact (apparent) pattern assumed being dependent on the perfection of the technical treatment. The essential result has been the fusion of the chromophobic material of the various chondriosome spheres to form a continuous, homogeneous matrix in which is embedded the chromophilic material at first in a fantastic, thread-pattern. How is this result to be compared with the nebenkern formed by the fusion of thread-like mitochondria?

Since it has not been possible to differentiate the chromophobic material in the thread-like mitochondria of the Hemiptera, one can not trace the gradual evolution of the nebenkern as Gatenby has done; although in preparations not too darkly stained one sometimes gets the impression that the substance producing the nebenkern is not as homogeneous as it often appears to be. Once fully completed (perhaps even earlier), the nebenkern begins to show the first clear signs of a differentiation into two substances. This first makes itself evident by the appearance of a large num-

[1] So far as I know, Gatenby's results have not yet received any confirmation from other workers. It is, therefore, possible that with further study of the nebenkern in Lepidoptera other interpretations of the "spireme" structure as described by Gatenby will be suggested. However, for the purposes of this discussion, I have considered Gatenby's account as correct, and the conclusions reached will not be affected in any important way should the details of the "spireme" formation fail of substantiation.

ber of small vacuoles in the periphery of the mitochondrial mass (Fig. 1). These are at first very indistinct and difficult to demonstrate, but they presently become clearer and form a complete layer investing the periphery of the nebenkern. They seem to stain little or not at all, in contradistinction to the central mass which is more darkly colored. This may be due to the fact that the process of differentiation is going on from without inwards, or it may be due to the optical superposition of several layers of vacuoles. I am rather inclined to think that the process of differentiation is going on throughout the whole mass, but that the peripheral portions perhaps progress more rapidly. It is a curious fact that in many Benda preparations which seem to be otherwise excellent, these and the later differentiation phenomena are not demonstrated at all. Further, I have not been able to get any preparations (of *Brochymena*) stained with sufficient sharpness to show these vacuoles as they should, of course, appear on the rounded surfaces of the nebenkern as one focuses up and down. Zweiger ('07), however, seems to have made out this stage in *Forficula* (see his Fig. 36), in which the whole nebenkern seems made up of very small vacuoles.

The process of differentiation by which these vacuoles are produced has doubtless been proceeding for some time, but is made visible only when the vacuoles attain dimensions within the reach of our technique. At all events, Fig. 1 represents only a transitory condition in a continuous process which is progressing toward a definite end. The successive figures which I have drawn are merely selected at convenient points along the way. Fig. 2 represents a stage somewhat later than the preceding figure, in which the nature of this vacuole formation has become much more evident. It is clear now that the formation of non-staining vacuoles is going on throughout the nebenkern mass, the outer ones apparently being differentiated more rapidly than the more interior ones. Thus it comes about that these vacuoles are arranged in rather definite layers which, in cross-section, would appear as concentric rings (Fig. 2).[1] The outermost layer clears

[1] This corresponds approximately to the period at which the centriole migrates from its originally anterior position (Fig. 1) to its definitive position at the base of the sperm head (Fig. 3). (See Bowen, '22.)

up very rapidly, the separate vacuoles fusing together to form large clear spaces separated from each other by septa which pass outward from the central mass to the exterior of the nebenkern, which is marked by a very definite membrane. The whole thing rather recalls the figure given by Meves ('00) of *Pygæra,* though no real comparison is, of course, possible.

It is probably the stages between Figs. 1 and 2 that have often been noted heretofore and likened to a "blackberry." It is clear that if, as often happens in the small-cell generations, the nebenkern as a whole took the stain rather heavily, its appearance would be something like that of a spherical mass of soap-bubbles —or a blackberry. The many bizarre figures of this condition would seem to indicate that fixation effects may play a rôle in determining the exact appearance of the final result. That the main features of this stage are real, is indicated, however, by its many reproductions in the literature of insect spermatogenesis. Thus, Henking's ('91) Fig. 63 and Paulmier's ('99) Fig. 43 are excellent reproductions of the "blackberry" stage in *Pyrrhocoris* and *Anasa* respectively, very similar to those which I have seen in *Murgantia.* The early stages in the development of the vesicles as seen in optical cross-section are clearly figured by Voinov ('03) in *Cybister* (Fig. 50), by Zweiger ('07) in *Forficula* (Fig. 37), and by Shaffer ('17) in *Passalus* (Fig. 19). Later stages in the condensation of the peripheral layer of vacuoles are figured by Holmgren ('02) in *Silpha* (Fig. 9*f*), by Gross ('07) in *Pyrrhocoris* (Fig. 98) by Stevens ('05) in *Blatella* (Fig. 150), and (probably) by Doncaster and Cannon ('20) in *Pediculus* (Figs. 20 and 21). However, it is not improbable that some of these cases are artifacts similar to those figured by Meves ('00) in *Pygæra* as mentioned above.

The clearing up of the outer layer of vacuoles now proceeds rapidly, the substance of the vacuole walls being apparently withdrawn into the underlying region of the nebenkern, and eventually the whole peripheral zone appears as a clear, non-staining envelope enclosing a central, chromophilic core (Fig. 3). Usually a connection or two is still retained between the external membrane of the nebenkern and the central portion, these connections

being apparently indeterminate in position. At the same time it becomes evident that the vacuolated condition of the central mass is likewise clearing up, the substance of the vacuole walls being condensed into concentric shells of chromophilic material marking the limits between the former concentric rings of vacuoles, while the substance of the vacuoles themselves flows together to form more or less continuous shells of non-staining material alternating with the thinner shells of chromophilic material. This arrangement is at first very incomplete and imperfect (Fig. 3), the chromophilic partitions being more or less irregular. Something of this intermediate period in the differentiation of the nebenkern is also figured by other workers, particularly by Wilke ('07 and '13) in *Hydrometra*. (See his Fig. 70 in *Hydrometra lacustris* ('07) and especially Figs. 77 and 74 in *Hydrometra paludum* ('13).)

At the period of Fig. 3, the shells of chromophilic substance are still joined at many irregular intervals by cross partitions. Presently, however, the pattern clears up considerably and becomes much more regular in its main outlines (Fig. 5). The concentric zones of material are now smoother and more nearly complete, and the nebenkern as a whole exhibits a tendency to a division of all the parts into two equal halves (Fig. 5). The general arrangement recalls the appearance of a hemi-sected "onion" —noted by so many students of insect spermiogenesis. Previous to this time the pattern seems to have been susceptible to the disintegrative action of acetic acid, but with the stronger development of the chromophilic plate-work, the pattern is very often preserved after almost any fixative. This stage has thus become the most familiar of the nebenkern "patterns." It was figured by von la Valette St. George ('86*a*) in *Blatta* (Fig. 74) and later described in detail by Henking ('91) in *Pyrrhocoris*. Wilke in *Hydrometra lacustris* ('07) (Figs. 72 and 75), and in *H. paludum* ('13) (Fig. 78) has figured this stage with what one is inclined to think a trifle too diagrammatic clearness. Attention may also be called to the figures by Stevens ('05) of *Stenopelmatus* (Figs. 84 and 85) and *Blatella* (Fig. 151), by Wassilieff ('07) of *Blatta* (Fig. 56), and by Boring ('07) of *Pœciloptera*

(Fig. 278). To other workers this stage has looked like a thread-work of some sort, an appearance due possibly to the disturbances of fixation or the failure to stain clearly (but compare also with Gatenby's description). Thus, compare Henneguy ('96) on the "filamentous" nebenkern in *Pyrrhocoris,* and other early workers. Many observers have also noted the curious disposition of the "layers" of the nebenkern to take the stain differently. This is particularly common after the osmic acid fixatives (Flemming), the results being often very fantastic and unexpected. Gross ('07) has figured a variety of such results in *Pyrrhocoris,* and I have seen much the same thing in the pentatomids. The most interesting of these phenomena is that of alternative staining, in which the different layers on one side of the mid-line are stained in various degrees while in the other half of the nebenkern the degrees of staining are exactly reversed. (See Gross ('07) Figs. 100. 151, 154, 155, and 156.) The meaning of these peculiar phenomena is entirely unknown.

The structural foundation of these patterns will be made somewhat clearer by a comparison of the nebenkern as seen from the side (Fig. 5) and from one pole (*i.e.,* in cross-section) (Fig. 6). It is evident, in the first place, that the substance of the nebenkern has become sharply differentiated into two substances of very different staining capacities. One of these stains little, or not at all, and forms a more or less homogeneous matrix in which is embedded a mass of material that takes the usual mitochondrial stains (crystal violet, for example). Further, this stained substance is arranged in very characteristic concentric rings, the successive rings being separated by zones of the non-staining matrix. These rings might possibly be taken for a tangled thread-work comparable to the "spireme" of Gatenby ('17), but by focusing up and down it is clearly proved that these rings are not threads at all but more or less extensive, shell-like plates. This is made even more clear by Fig. 6 in which the nebenkern is cut at right angles to that of Fig. 5. It is quite obvious that the apparently concentric rings are in reality the optical cross-sections of a more or less complete plate-work, of which the imperfectly spherical plates are arranged one within the other in a concentric

series. *The patterns are merely optical cross-sections of a plate-work.* It is difficult to be sure of this plate-work when the nebenkern is viewed "in the round," but I have tried to represent a small tangential slice of it in Fig. 4. The plate is more or less wrinkled and apparently perforated in places, which irregularities seem to correspond with the points of indentation, folding, or union, of the various plates, as can be explained by a comparative study of Figs. 4, 5 and 6. In the Hemiptera, therefore, a spireme seems not to occur at this stage, and the patterns are due not to a tangled thread-work but to a more or less regular plate-work.

It is necessary now to retrace our steps to the early stages in the formation of the nebenkern, and attempt an explanation of these results from a broadly comparative standpoint. It will be recalled that the peculiar pattern in the lepidopteran nebenkern owes its origin to the arrangement of the chromophilic and the chromophobic constituents of the original granular mitochondria. The question then arises: are the patterns just described in the Hemiptera comparable in any way to those in the Lepidoptera? I believe that all the facts point to an affirmative answer to this question.

It will be recalled that from a comparison of various morphological types of mitochondria the assumption seemed to be warranted that they are all alike composed of two fundamental and distinct substances—a chromophilic material forming the outer covering of each chondriosome unit, and an internal chromophobic material. Assuming this structure to be present in the thread-like type of mitochondria, the transformations of the hemipteran spermatid are readily explicable. During the early stages in the formation of the nebenkern, the presence of the two mitochondrial materials can not be discerned any more than was possible in the auxocyte period. It is, therefore, impossible to say exactly how the fusion of the threads takes place, as Gatenby has done in the case of the vesicular mitochondria. It seems probable, however, that, as in the latter case, there is a process of running together going on within the chondriosome mass, which finally results in the production of sufficiently large groupings of the two materials to allow of a visible separation. This condition is at-

tained in the stage of Fig. 1, in which the chromophobic material has run together to form many small, clear masses or vacuoles, the walls of which are formed, as it were, of the chromophilic substance. The process of condensation which has produced this initial differentiation now continues rapidly (Fig. 2), larger and larger aggregates of chromophobic material being produced by the further condensation of the chromophilic substance. This condensation has seemed, from the beginning, to progress from without inward, so that the primary vacuolization is in the form of concentric shells of vacuoles, each equal in thickness to the diameter of a single vacuole. Thus by the absorption of the partitions of chromophilic material which at first separate the vacuoles of any one layer, there is produced a very typical figure. The chromophilic material has accumulated in a thin lamella between each adjacent layer of vacuoles, while the vacuoles themselves have run together to form a more or less homogeneous ground substance which surrounds, and fills in all the interspaces between the chromophilic lamellæ. These lamellæ seem to be more or less inter-connected and wrinkled, and are possibly perforated in numerous places, producing a sort of skeletal plate-work, the main outlines of which are molded on the original vacuolization of the nebenkern. The "spireme" of Gatenby ('17) and the "plate-work" which I have described are thus directly comparable in every way. Their apparent difference depends, it would seem, on the original form and method of fusion of the spermatid chondriosomes, which are in one case large, vesicular granules and in the other long, delicate threads.

I believe, therefore, that, depending on the primary morphology of the auxocyte chondriosomes and possibly other unknown factors, the nebenkern may develop a "spireme" structure or a system of plates (or possibly other structural types not yet described). Gatenby ('18) on the other hand seems inclined to the view that all the nebenkern structures are essentially a thread-work comparable to the "spireme" in Lepidoptera. He states, in fact, that a "very finely-coiled spireme" does occur in *Tenebrio,* and presumably other Coleoptera. It is always possible, as Gatenby has shown in the Lepidoptera, that faulty technique will

make one of these types look like the other. This may possibly have occurred with some of the Orthoptera, for I find in my own material of *Rhomaleum,* nebenkern figures comparable to those figured by Meves ('00) in *Pygæra,* and yet Giglio-Tos and Granata ('08) figure in *Pamphagus* a very clear "spireme." Of even more interest are the old figures of the nebenkern in *Gryllus* by Baumgartner ('02), in which a "striated condition" very reminiscent of Gatenby's "spireme" is a conspicuous figure. It is, therefore, by no means improbable that the "spireme" type of nebenkern is more widely distributed than has been suspected. On the other hand I am equally convinced that the "plate-work" type of nebenkern is a reality, and it is scarcely probable that technique of so many kinds (I have tried a great many methods on these Hemiptera (see Bowen ('22))) would fail to reveal a "spireme" in the hemipteran nebenkern if any such structure really existed. The whole series of stages which I have described are hardly to be satisfactorily explained away as artifacts. It is, I think, safe, then, to conclude that there are at least two fundamental types of nebenkern structure—the "spireme" type, and the "plate-work" type—possibly interconnected by intermediate conditions; but until the matter has been carefully examined it will not be possible to classify the various insect groups (other than Lepidoptera and Hemiptera) on the basis of nebenkern type. This whole subject offers an attractive field for intensive study.

It remains now to follow out the later history of the chromophilic substance. In the spireme type the chromophilic thread often withdraws from its originally peripheral position, and, after imperfect fixation, the running together of its substance gives rise to the appearances figured by Platner ('89) and Meves ('00) and already referred to above. The final fate of the spireme is not known in any case. In the Lepidoptera the nebenkern does not divide (according to the current descriptions) as it does in the Hemiptera and many other insects, but merely draws out along the tail filament for which it acts in the rôle of a mitochondrial sheath. According to Gatenby ('17), as this elongation is going on, "the chromophobe substance 'dwindles,' and the spireme ap-

pears to break up partially." In this unsatisfactory manner the account closes. The probable fate of the spireme will be touched on in a later paragraph.

To return now to the plate-work in the nebenkern of Hemiptera. The process of condensation having reached the stage of Figs. 5 and 6, the plate-work begins now to become much more open and irregular; and in the immediately succeeding stage the resemblance to an onion has been largely lost (Figs. 7 and 8). The running together of the chromophilic material has resulted in the disappearance of a part of the plate-work, which now consists of a few much-folded plates whose optical cross-sections are usually rather simple. Fig. 7 is an especially striking one, since in this case the outline forms a perfect spireme reminding one of Gatenby's account. Fig. 8 is another example of the same stage, and by changing the fine adjustment slightly, the optical section can be converted into a perfect spireme. By a similar change of focus, the spireme of Fig. 7 can be transformed into an appearance similar to that of Fig. 8. If further proof of the plate-like rather than thread-like structure is necessary, it can be provided by a cross-section of the nebenkern (Fig. 9). (Figs. 8 and 9 are from the same cyst of spermatids.) Comparing this cross-section with Fig. 6, it is clear that the chromophilic substance is still arranged in a plate-work form, but now a very irregular one. It is also of interest to note that the plate-work seems to end at opposite points on the surface of the nebenkern, thus marking the future division plane of the mass as a whole.

At a slightly later stage (Fig. 11) the plate-work has become still more condensed and simplified, while the chromophilic substance of which it is composed seems to be decreasing in actual quantity. At this time, as is shown also by cross-sections (Fig. 12), the plate-work forms a flattened, ovoidal core, the exterior of which is now delimited by a smooth, regular layer. Internally a single, much-folded septum is inserted in this outer layer along the future division plane of the nebenkern. The further condensation of the plate-work now progresses rapidly, the ovoidal core of chromophilic substance shrinking considerably in size, while the folded, internal septum smoothes out to form a flat, regular

partition (Fig. 13). If the spermatid be viewed at right angles to the plane of division of the nebenkern (*i.e.*, at right angles to the plane of Fig. 13), the central chromophilic mass appears as a single, regular plate (Fig. 14).

Slightly later the chromophilic core has shrunk still further (Fig. 15) and finally it becomes so much reduced as to be barely visible as a purple-staining (after Benda) patch within the nebenkern proper (Fig. 16). As the nebenkern begins actively to elongate, the last remnant of the chromophilic material vanishes (Fig. 22) and no trace of it can be found at any subsequent stage. It has disappeared apparently by a process of gradual solution in the chromophobic substance.

The gradual breaking down and disappearance of the "onion" stage has been noted by several previous workers; and still others have figured several of the later stages without apparently any very accurate idea of what was occurring. Henking ('91) described the process in *Pyrrhocoris,* the layers of the onion stage gradually disappearing from without inward by fusing together, leaving at length a small, dark point in the center of the nebenkern which likewise disappears. Gross ('07), working on the same form, has given a slightly different account, less accurate in some respects than that of Henking. Boring ('07) has clearly figured (Figs. 304 and 313) the intermediate condition of my Figs. 7 and 8 in *Amphiscepa* and *Pœciloptera,* while the very regular arrangement of the chromophilic substance in its later phases of dissolution is well figured by Doncaster and Cannon ('20) in *Pediculus.* It is of interest in connection with these last named observers, that they made a conscious effort to find a "spireme" like that described by Gatenby, but failed entirely. Their figures indicate a course of events similar to that in the *Pentatomidæ.* Many other workers have figured what appear to be these later stages in the history of the chromophilic substance, but these need not be dwelt upon here since it is uncertain whether they are to be considered as the end stage of the plate-work type of nebenkern or an artifact from a spireme structure. These figures have the common characteristic of a clear peripheral envelope containing a chromophilic central mass. (See, for example, the

figures of Holmgren ('02) (*Silpha*), Vejdovský ('12) (*Diestrammena*), Shaffer ('17) (*Passalus*), and Zweiger ('07) (*Forficula*).)

One further feature which seems to have some relation to the final disappearance of the chromophilic core remains to be considered, viz., the division of the nebenkern into two parts. It will be observed (Fig. 13) that the nebenkern commences to elongate before the chromophilic substance has entirely disappeared, and at the same time there is a marked indication of the longitudinal splitting of the nebenkern into two parts. This division plane seems to deepen as the chromophilic core withdraws more and more toward the center of the nebenkern (Fig. 15), but the division never extends through the core itself. This is clearly shown in Figs. 21*A* and *B*, which show optical cross-sections of the nebenkern at about the stage of Fig. 15, at two different levels. In Fig. 21*A*, the section passes through the chromophilic substance, while in Fig. 21*B* it passes above (or below) it. It is clear that the actual division of the nebenkern has extended only to the periphery of the central, chromophilic mass. When this finally disappears, the division of the nebenkern is completed (Fig. 22), and from this point on the nebenkern draws out as two separate bodies—the mitochondrial sheaths of the tail filament—as described by many authors. It is interesting to note that Henking ('91) observed, after the final condensation of the "pattern," that the two halves of the nebenkern became more and more *sharply set apart* from each other. Thus it appears that the division of the nebenkern waits upon the final disappearance of the chromophilic material, the completion of which process signalizes the final splitting of the nebenkern into two parts.

If there is any real connection between these two phenomena, we should always expect the disappearance of the chromophilic material before the division of the nebenkern. Accordingly, if the absorption of the chromophilic substance is for any reason delayed beyond the initial phases in the drawing out of the nebenkern as a whole. we should expect the nebenkern to elongate as a single mass dividing eventually into two parts at a stage in the elongation dependent upon the time of the disappearance of the chromo-

philic core. The first of these expectations receives a remarkable fulfillment in many insect forms, and I am not aware that the sequence is ever certainly violated. The Lepidoptera, as described by Meves ('00) and Gatenby ('17) are particularly good examples, and many Coleoptera exhibit a similar condition. Thus, in *Silpha,* Holmgren ('02) figures very clearly the gradual disappearance of the chromophilic material as the undivided nebenkern draws out, and in cross-sections very similar to those which I have figured, he shows that the division furrow of the nebenkern extends only to the periphery of the chromophilic core (his "Mitochondrienrestkoerper"). Shaffer ('17) figures a similar stage in *Passalus,* which he seems to think is divided. From the position of the nebenkern parts, I think, however, that the conditions are like those in *Silpha,* Shaffer having confused the indications of division with the accomplished fact. Still other cases might be cited, all of which tend to support my contention that the division of the nebenkern is never completed until the chromophilic substance has disappeared. It remains, then, to inquire whether, in these cases of delayed division, the disappearance of the chromophilic material is followed by the definite splitting of the nebenkern. The evidence on this point is very scanty since, if division is long delayed, it would usually be overlooked. However, in the case of *Silpha* (Holmgren ('02)) the whole history of events is clear, and Holmgren's figures show conclusively that the nebenkern completes its division after the disappearance of the chromophilic substance. In the case of *Passalus,* if my interpretation of Shaffer's Fig. 21 be correct, the nebenkern is subsequently divided, as shown by his Fig. 20*c,* though the exact relation to the disappearance of the chromophilic material is not established. The classical case of the lepidopteran nebenkern has never been followed out in detail, and it would seem that the chromophilic material retains its identity for a long period. But I will venture the guess that in this case, also, a division of the nebenkern will be found to occur once the chromophilic substance has been disposed of.

This completes the history of the origin and nature of the "blackberry" and "onion" patterns which were mentioned in the

beginning of this paper as two characteristic types in the insect nebenkern. It remains now to consider the nature of the third type of pattern—the smooth or beaded cords running parallel to the long axis of the nebenkern as mentioned in the paragraph referred to above. This type of pattern has been described especially by Holmgren ('02) and Vejdovský ('12). Fragmentary references to it occur, however, in a number of other cases, as for example those of *Pygæra* (Meves ('00)) and of *Notonecta* (Pantel and de Sinéty ('06)); Henneguy ('96) also seems to have figured it crudely in *Pyrrhocoris,* and Boring ('07) shows it very clearly in *Pœciloptera.* In so far as any explanation of this pattern has been attempted, all accounts agree in connecting it ultimately with the preceding patterns, that is, with the chromophilic substance. This is certainly not the correct origin in the *Pentatomidæ;* in fact, we have here to deal with a structure of entirely independent (so far as its morphology is concerned) origin. Indeed, it is better to consider this from the standpoint of the evolution of a new substance within the nebenkern, rather than as a mere pattern comparable to those which have preceded it.

This substance, then, makes its first appearance at a period approximately that of Figs. 7, 8 and 9—in other words at about the time when the chromophilic substance first seems not only to be condensing but actually in process of dissolution. This new material appears at this time in the form of vacuoles scattered about in the chromophobic substance between the chromophilic plate-work and the peripheral wall of the nebenkern (Figs. 7, 8 and 9). These vacuoles are rather inconspicuous at first, their periphery staining faintly in Benda preparations, though I am not able to say positively whether the alizarin or the crystal violet is responsible for the coloration. It seems to be the former as a rule. The origin of these vacuoles is entirely obscure. Perhaps they are produced as a special differentiation of the chromophobic material. However, the fact that these vacuoles are on the increase while the chromophilic material is decreasing suggests that there is possibly some connection between the two processes. It might be supposed that the vacuoles are differentiated out of material derived from the chromophilic mass in some indirect way.

Whatever the origin of these vacuoles may be, they increase rapidly in number, and soon become arranged in thread-like rows running parallel to the future long axis of the nebenkern, subsequently becoming more or less fused together to form structures reminding one of strings of beads (Fig. 11). In cross-section, these threads appear as single vacuoles, the position of which marks the distribution of the threads (Fig. 12). In immediately subsequent stages (Figs. 13 and 15) these beaded threads become still more conspicuously developed, and as the chromophilic substance disappears they become distributed throughout the chromophobic envelope as shown by cross-sections of the nebenkern (Figs. 21*A* and *B* and 22).

The account, thus far, is taken from Benda preparations, in which the vacuoles stain faintly as described above. Occasionally, however, the substance itself of the vacuoles stains more or less darkly with the crystal violet (right half of Fig. 11), becoming much more conspicuous than is usually the case. Very frequently these vacuoles are not demonstrated at all by the Benda method, the chromophobic material appearing entirely clear. The above account applies particularly to the *large-cell generations;* in the *small-cell generations* (in *Murgantia* the large-cell generations also are not sufficiently large to make any difference in this respect) the results with Benda are quite different. In these the crystal violet often acts rather capriciously, but as described by many authors it usually *stains the whole mass* of the nebenkern an intense violet. In other words, the *chromophobic* material takes the *violet stain.* This difference in staining behavior of the large and small nebenkerns, so fortunate for this investigation, is probably due to some physical factor which results in the ready extraction of the stain from the large nebenkern masses while from the small ones the stain is usually extracted with more difficulty. In the latter case, as in the former, the substance of the newly formed vacuoles fails to take the violet stain in good preparations, and thus the substance of the nebenkern appears to be composed of two materials of very different staining capacities. I thought, at first, that the material which thus takes the crystal violet (in the small-cell generations) was

equivalent to the outer, chromophilic substance of the original mitochondria;[1] but the history which I have traced shows clearly that exactly the reverse is the case. So much for the dangers of arguing on the basis of a single staining reaction. As a result of this behavior toward crystal violet, the nebenkern appears vacuolated in various characteristic ways, the vacuoles being at first scattered, and subsequently arranged in longitudinal rows that seem to be fusing to form a non-staining axis for the outer, intensely staining substance. (See Bowen ('22) Figs. 59, 60 and 66.)

The most interesting results, however, were obtained with Cajal's formol-uranium nitrate method with which I had been attempting the demonstration of the Golgi elements. This technique gave no certain results in the very early stages of the formation of the new, or as we may conveniently refer to it, the *central,* substance, but when the vacuoles had lined up to form thread-like chains (about the stage of Fig. 11), the silver was very heavily reduced by the substance of the vacuoles themselves which were thus brought out with the clearness of a silhouette on a field of bright canary yellow (Fig. 10). The general fixation of the spermatid is usually very poor, the nucleus appearing merely as a light, circular area, often badly distorted. But the nebenkern is well preserved and the beaded threads stand out with remarkable clearness, especially in later stages. At this early period they are not quite so clear on account of the mass of chromophilic material which still occupies the center of the nebenkern and is colored a nondescript brown. Whatever may have been the origin of the substance of these threads, it is abundantly clear that it represents now a material of specific and characteristic chemical composition. I had thought at first (Bowen ('19)) that it represented the original chromophobic material of the chondriosomes, but it is now evident that such can not be the case.

As the nebenkern draws out, the threads make for a time a rather confused picture since the sections rarely cut them exactly parallel to their length (Fig. 17 is a fair example). The cross-sections of these stages are more interesting, the threads, now cut

[1] It should be remembered that the true chromophilic substance disappears entirely at a relatively early stage, as described above.

across, appearing with diagrammatic clearness (Fig. 20). In early stages I have counted at least 15 to 20 such thread sections in a single half of the nebenkern. At this same stage Kopsch preparations are sometimes obtained in which the chromophobic substance is very intensely blackened while the threads are left clear. Thus a picture exactly the reverse of the Cajal result is obtained. (Compare Figs. 17 and 20 with Figs. 18 and 19.) With the elongation of the nebenkern the threads spin out along its course and show a progressive decrease in number, as can be clearly demonstrated in cross-sections (compare Figs. 20 and 24), as well as in plane views (Fig. 23) of the nebenkern. They now show the beaded appearance perhaps due to their origin from chains of separate vacuoles, in a very characteristic manner. It is not easy to say at this stage just how the reduction in the number of threads takes place, but there appears to be a side by side fusion of adjacent threads, a single thread thus arising from two preceding ones. This fusion goes on rapidly until at the stage when the tail vesicles (see beyond) are first formed, the threads of each half of the nebenkern have fused to form a single axial core for their respective nebenkern portions (Figs. 25 and 26). In the final condensation of the threads proof of the lateral fusion of the separate threads can be obtained; for in stages just prior to that of Fig. 26 the threads will, in some places along their course, be already fused to form a single axial core, while in other places they are still clearly separate. Paulmier ('99) noted this central core in the elongating nebenkern halves of *Anasa,* likening it to "a long vacuole extending down each one."

The origin of these threads (central substance) seems to have been studied only by Holmgren, and Vejdovský. In *Silpha,* according to Holmgren ('02) there is, in each half of the nebenkern, a single, beaded thread, which arises from the central chromophilic mass by a process of splitting off. To these thread-like portions thus separated off is given the name of "Mitochondrienbalken." Slightly later the chromophilic mass disappears as I have already noted above, and the threads now occupy the halves of the divided nebenkern exactly as I have described the arrangement in Hemiptera. Holmgren figures also a number of cross-

sections which are very similar to mine, except that there is never more than one thread in each half of the nebenkern at any stage. Subsequently the chromophobic material is said to disappear and the threads themselves finally dissolve leaving no trace of mitochondrial substance in the mature sperm. This account of the fate of the threads and the nebenkern substance as a whole is negatived by more recent studies on the rôle of the mitochondria in sperm formation. As to the mode of origin described by Holmgren, it seems to me not improbable that technical faults caused him to overlook the early history of the threads, and at the time when he first demonstrated them their development was already well advanced.

In *Diestrammena,* according to Vejdovský ('12), the nebenkern has at first the characteristic division into a central chromophilic substance and an enveloping zone of chromophobic material. Cross-sections of this stage are quite similar to mine except that the axial filament is represented as piercing the center of the chromophilic core. This is a very unusual condition, if true, the customary position of the filament being in or near the cleft of the nebenkern, but outside of the substance of the nebenkern itself. (Compare my Figs. 21*A* and *B,* and 22 with Vejdovský's Figs. 181*e, f, g.*) Presently there appears in the chromophilic material a number of separate, intensely staining granules which become arranged in rows parallel to the axial filament; and eventually the chromophilic material is entirely replaced by spirally wound (later beaded) threads. The end result is strikingly like that in the Hemiptera. Vejdovský believes that the process is to be interpreted as a transformation of the "Chondriom" (chromophilic substance) into the granular components ("Mitochondrien") from which it originally arose. According to this view the threads would be secondarily a differentiation product of the chromophilic material itself—a view somewhat similar to that of Holmgren. The beaded appearance of the threads marks, according to Vejdovský, the breaking up of the threads, the granular products (Mitochondrien) thus formed swelling up to a large size and being simultaneously transformed into a fat-like substance. These fat droplets seem to fuse together to form larger

aggregates, at the same time *absorbing the chromophobic material,* with the final result that the axial filament lies naked in the cytoplasm with the fat droplets scattered along its course. These later pass out along the tail to be collected in the protoplasmic masses that are sloughed off in the final maturing of the sperms, which, as a result, possess no trace of mitochondria or their derivatives. This again reminds one of Holmgren's account, and the same comment relative to the findings of other authors is applicable by way of criticism.

In view of the unusual fate ascribed by Holmgren and Vejdovský to these final structural components of the nebenkern, it is of interest, finally, to inquire into their later history in the Hemiptera. In stages subsequent to that of Fig. 26 the Cajal method fails to impregnate the central substance of the mitochondrial sheaths, which continue to draw out rapidly with a consequent decrease in their diameter. The only evidence which I have been able to get on the structure of the nebenkern in these later stages has been obtained from Benda preparations. I have already called attention to the fact that in *some* cases (*e.g., Murgantia*) I have obtained preparations in which the outer, chromophobic material stained intensely with crystal violet, while the substance of the vacuoles (the central substance) remained colorless. Accordingly, when the substance of the latter has run together to form a single axial core for each half of the nebenkern (Fig. 26), the two substances can still be distinguished, the outer layer (the chromophobic portion) staining a deep purple while the central core (the central substance) is unstained. (See Bowen ('22) Figs. 76 and 78.)[1] As I have described in another paper (Bowen, ('22)) the mitochondrial sheaths begin now to develop character-

[1] It may be as well at this point again to call the attention of the reader to the confusing differences in the staining behavior of the nebenkern substances. In the first place, it is to be noted that the *central substance* almost invariably fails to take the crystal violet of Benda's method, while the *chromophilic material* is always stained violet if the nebenkern retains the stain at all. The *chromophobic material,* however, behaves in a variable way. Under the best conditions it does not retain the crystal violet (at least in early spermatid stages) when the rest of the preparation is properly differentiated; but sometimes in the early spermatid stages (small-cell generations), and invariably in the later ones, it stains heavily with the violet—*an exact reversal of staining behavior.*

istic vesicles, the intervening substance being simultaneously spun out into thin connecting threads. The result (Fig 27) is that each of the sheaths becomes spun out into a long, delicate thread along the course of which these vesicles are scattered as bleb-like swellings. The structure of these swellings is very clearly demonstrated by cross-sections of the tail. In such sections (Fig. 28) the sheaths themselves appear as small dots intensely stained with crystal violet, while the vesicles appear as masses of non-staining material enclosed by a peripheral layer which stains like the portions of the sheaths which intervene between two adjacent vesicles. It is as if the thread-like sheaths were inflated at intervals to form little pockets in which is placed a small mass of some material staining quite differently from the substance proper of the threads. My impression is that these vesicles are eventually smoothed out, their substance being perhaps distributed evenly along the thread-like mitochondrial sheaths. Needless to add these sheaths are not broken down as stated by Holmgren and Vejdovský. (See Bowen ('22)). It is also clear that it is these vesicles which Vejdovský has confused as the "fat droplets" derived from the breaking down of the mitochondria as a whole. Obviously, their real nature is entirely different.

The exact origin of these vesicles has not been satisfactorily made out, and the relation of their structure to that of the earlier stages is therefore uncertain. It is possible, however, that the central substance of the nebenkern derivatives tends to collect at intervals along the course of the sheaths, thus forming the bleb-like swellings, while in the intervening lengths, from which the central substance is withdrawn, the sheath is correspondingly thinned out (Fig. 27). Possibly such accumulations of material play some rôle in the spinning out of the sheaths. The ultimate fate of this central substance is unknown; possibly it comes finally to form a delicate core for each of the thread-like mitochondrial sheaths. Thus is completed the long and complicated differentiation of the spermatid nebenkern.

Conclusion.

One of the most interesting results of this study has been to reveal the nebenkern as the seat of an unexpectedly complicated

and determinate series of differentiations. Instead of an inert, passive mass of mitochondria subject only to some enigmatic structural "patterns," we find that the nebenkern is actually the seat of intense activity up to a very late stage in sperm formation. What the meaning of this may be, is, however, entirely unknown at present.

A second point of very practical nature is the light thrown upon the staining behavior of the mitochondrial substance during spermiogenesis. It is clear that in preparations which have every appearance of technical excellence, the structure of the nebenkern may be seriously distorted or entirely invisible. This has obviously been the cause of many incomplete accounts, and of much contradiction of results even in the same animals. It now becomes plain that the exact study of the nebenkern demands the use of a great variety of technical methods in the hope that one of them will prove adequate for the work in hand. Similarly, the obvious changes in the chemical composition of the nebenkern substance arouse a well-grounded skepticism as to the value of the so-called "specific" stains for mitochondria. In a mass of changing chemical composition such as I have shown the nebenkern to be, it is manifestly impossible to talk of any "specific" staining at all; and equally impossible to assign a chemical composition to this chondriosome body as one might to a homogeneous mass.

I have tried in this paper to put into a logical and connected story the facts as we know them about the spermatid nebenkern in insects. In no single form have all the features of this account been, as yet, clearly made out, and my interpretation of the facts as I have found them in the Hemiptera involves, therefore, a large element of assumption. I have, however, felt justified in these assumptions since they furnish a satisfactory basis of explanation for all the facts so far available, many of which have been entirely obscure hitherto; and since, furthermore, they provide a standpoint from which a further, intelligent attack on the whole problem can be made.

By way of summary, attention may be particularly called to the following points:

1. The nebenkern of the insect spermatid is composed primarily of two distinct substances—a chromophobic ground-substance presumably equivalent to the medullary material (of like staining behavior) in the original chondriosomes; and a chromophilic substance presumably equivalent to the peripheral material in the original chondriosomes.

2. The chromophilic substance is arranged in a variety of ways producing the so-called nebenkern "patterns."

3. These patterns are presumably of at least two types—(1) the "spireme" type characteristic of Lepidoptera, and (2) the "plate-work" type characteristic of Hemiptera.

4. The chromophilic substance eventually disappears apparently by some process of condensation and solution.

5. As the chromophilic substance disappears, a new substance, possibly a differentiation product of the chromophilic material, makes its appearance within the mass of chromophobic stuff.

6. This new (central) substance forms a core for each of the elongating halves of the nebenkern, and possibly is the source of the bleb-like swellings later developed on the tail sheaths.

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EXPLANATION OF PLATES.

All of the figures have been outlined as far as possible with the camera lucida at an initial enlargement of approximately 3,800 diameters. At so great an enlargement it has, of course, been necessary to correct the outlines extensively and to add much of the finer detail free hand. In reproducing, the figures have been reduced uniformly to an enlargement of approximately 3,000 diameters. In every case the method employed in the preparation of the original object has been indicated.

PLATE I.

Fig. 10 is from *Euschistus euschistoides;* the others are from *Brochymena quadripustulata.* All the figures are from cells of the large generations.

FIG. 1. Early spermatid with first indications of nebenkern differentiation. (Benda.)

FIG. 2. Slightly later stage than the preceding, showing development of the peripheral, chromophobic zone. (Benda.)

FIG. 3. Peripheral, chromophobic zone completed and early differentiation of the chromophilic plate-work. (Benda.)

FIGS. 4, 5, 6. Intermediate stage in the development of the chromophilic plate-work. Fig. 4 is a surface view of a small slice of the nebenkern plate-work. Fig. 6 is a cross-section of the nebenkern at right angles to the major axis of the spermatid, the axial filament appearing below in cross-section. (Benda.)

FIGS. 7, 8, 9. Early stage in the final condensation of the chromophilic plate-work and the first appearance of the vacuoles (central substance) in the chromophobic material. Fig. 9 is a cross-section of the nebenkern made like Fig. 6, the tail filament appearing below. (Benda.)

FIG. 10. Early stage in the running together of the vacuoles in the chromophobic material to form beaded threads. (Cajal.)

FIGS. 11, 12. Slightly later stage than the preceding, showing intermediate stage in the final condensation of the chromophilic material. Fig. 12 is a cross-section of the nebenkern made like Figs. 6 and 9. The beaded threads are seen in the chromophobic material. (Benda.)

FIGS. 13, 14. Later stages in the disappearance of the chromophilic plate-work. Fig. 14 is at right angles to the plane of Fig. 13. (Benda.)

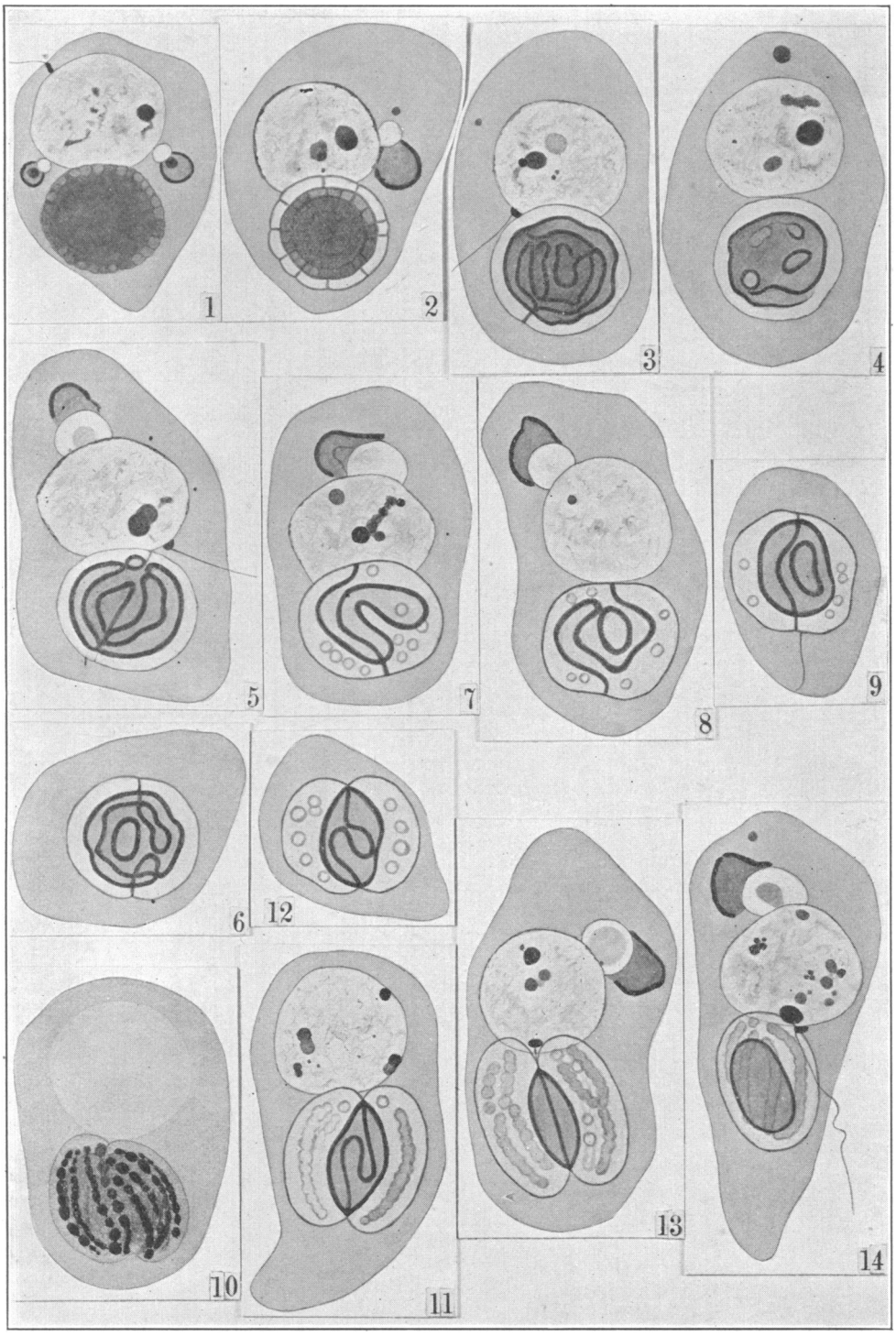
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PLATE II.

Figs. 27 and 28 are from *Murgantia histrionica;* Figs. 15, 16, 21*A*, 21*B* and 22 are from *Brochymena quadripustulata;* the others are from *Euschistus euschistoides*. All the figures are from cells of the large generations.

FIG. 15. Late stage in the disappearance of the chromophilic substance. (Benda.)

FIG. 16. Final stage in the disappearance of the chromophilic substance. (Benda.)

FIG. 17. Early stage in the drawing out of the nebenkern, showing the vacuoles and beaded threads (central substance) which have recently appeared in the chromophobic substance. (Cajal.)

FIGS. 18, 19. Same as Fig. 17. Fig. 19 is a cross-section of the nebenkern at right angles to the major axis of the spermatid. (Kopsch.)

FIG. 20. Cross-section of the nebenkern made like Fig. 19, at a stage slightly later than that of Fig. 17. (Cajal.)

FIG. 21. Cross-sections of the nebenkern made like Fig. 19, approximately at the stage of Fig. 15. *A*, through the level of the chromophilic plate-work; *B*, above (or below) the level of the chromophilic plate-work. (Benda.)

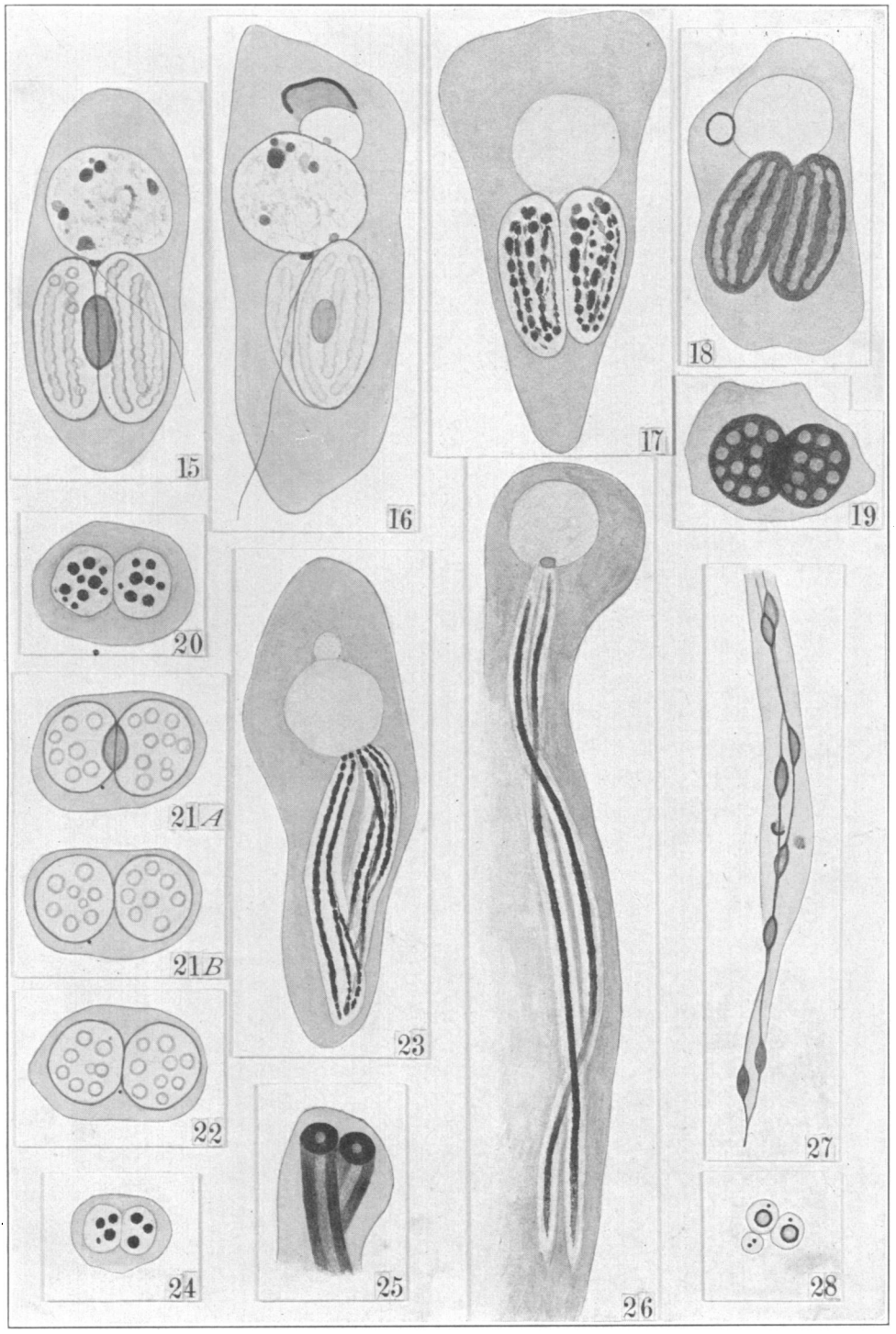
FIG. 22. Cross-section of the nebenkern made like Fig. 19, after the final disappearance of the chromophilic substance. (Benda.)

FIGS. 23, 24. Intermediate stage in the lateral fusion of the threads of central substance. Fig. 24 is a cross-section of the nebenkern made like Fig. 19. (Cajal.)

FIG. 25. Portion of the mitochondrial sheaths at about the stage of Fig. 26. At one end the sheaths were turned upward and so cut in cross-section. (Kopsch.)

FIG. 26. Final stage in the condensation of the central substance; the threads of Fig. 23 have fused to form a single, axial core for each of the nebenkern halves. (Cajal.)

FIGS. 27, 28. Development of the bleb-like swellings on the mitochondrial sheaths. Fig. 27 is a small portion of the tail region near the head of the sperm. Fig. 28 is a cross-section of three sperm tails at the stage of Fig. 27. (Benda.)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